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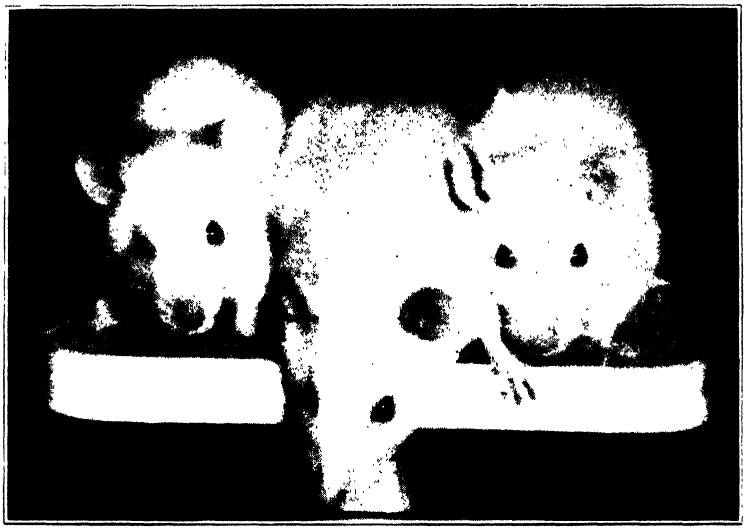
LABORATORY EXPERIMENTS *in* NUTRITION



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PREFACE

Opportunity for animal experimentation has become commonplace even for the small school with a limited budget for equipment. The modern biology teacher believes in demonstrating the fundamental principles in his science and into the discard has been thrown the old idea of teaching biology by textbook alone. The progressive biology teacher makes all of the out-of-doors his laboratory at will, or brings into his own laboratory that from the field which can be maintained for class observations. A far cry, this idea applied to nutrition studies, from the antiquated textbook recitation alone. In view of this, we ask: "How can boys and girls be expected to remember facts so abstractly presented to them? Combining the abstract study with *actual nutrition experiments* will drive home these concepts so that many of them will be remembered for life. The mental pictures of "deficient" rats are easily acquired during this impressionable age period and are not easily erased, even by decades.

In this booklet we have not endeavored to tell you how to teach the subject of nutrition—there are excellent treatises on this subject already. Rather, we have striven to give all of the practical, concise information necessary to demonstrate effectively and inexpensively the value of the various basic constituents of foods. As this booklet was being written we were mindful of the grammar school teacher, who wants to conduct, say, only one or two of the simpler experiments, the high school biology teacher who has always wanted somehow to verify with his students the facts concerning the nature and effects of various food substances but who never knew just how to go about it, and the college biology teacher who wishes to have his students make careful and exacting tests along all lines presented here as well as many specific avenues of research that will be suggested as the experiments go on to completion. It must, therefore, serve a rather wide range of teaching requirements and we are quite cognizant of the fact that the somewhat detailed accounts of certain procedures, though all important for the inexperienced of our readers, are not required for those who are conversant with experimentation in this subject.

It should be emphasized that our effort here is primarily to present the side of the subject which will be of the most good to the *biology* teacher. Specialized courses in nutrition for colleges already have their texts and it is not our purpose to invade this field. It has been our experience, however, that *biology* teachers wish to use the material here presented in that unit of their work relating to nutrition or food study.

Much of the information in regard to the general care of the experimental animals is the result of our own experiences with them. These have been supplemented, however, by actual observations made specifically for us by one of the largest commercial breeders of white rats in the country. In addition to breeding this form this firm also makes a specialty of vitamin assay work and countless practical suggestions are the outcome —many of which have been incorporated here. Where possible, we have used original formulae for the experimental diets—formulae which have been tried over and over again and are known to have the desired effect. Some formulae are not original with us, the original worker to use each has been mentioned in connection with it, and we claim no credit for such.

This first edition has been printed in a comparatively small number, it being anticipated that there will be many more points of interest brought to our attention by teachers and these we shall include in future editions. We would like you to consider even this booklet experimental—subject to modification as you may suggest. The questions asked by teachers will determine to a large extent the revisions to be made in a subsequent edition. Do, therefore, feel that you are cordially invited to participate in making this a complete and *practical manual for the nutrition unit in the biology course.*

Chapter I

INTRODUCTION

One of the primary aims of all education is to prepare the student for living a healthful and happy life. So frequently as we look back upon the past educational procedures we see that generally the subject matter received first consideration and the good that this might do the individual student was of more or less incidental importance. Modern education is to be commended upon centering the primary attention upon the individual and adapting and shaping the subject matter so that it can best be synthesized by the student into a background of knowledge he may call into use as situations demanding it arise.

Quite naturally it follows that in a modern curriculum due emphasis is to be placed upon health education. If we inquire into it we see that this subject is not deferred until the later years of youth but instead is instituted in the lower grades where the preliminary concepts are most profitably given. Correct health habits must be started when the child is young—when preventive measures will preclude the necessity of remedial measures later. Rose tells us that: "Ten years of study of the teaching of nutrition in all grades from kindergarten to the eighth, has made it quite evident that the first level at which some clearly formulated nutrition principles may be most profitably developed with children is about the fourth or fifth grade. This is the time when children begin to be interested in the control of their own lives and also when they are often given increasing latitude in the choice of their own food."

Many of the diseases due to dietary deficiencies would practically disappear if even this early training in nutrition could be practiced universally. But we would not stop with the early training, important though it be. In high school we would continue it and this can surely be done *in the biology course* where the broader principles are introduced —nutrition as applied to all living things, plant and animal. Make the boy and girl the subject of investigation and study their nutritive processes. It is obvious that to completely satisfy their questions animal experimentation must be used. Let them conduct certain of the experiments mentioned in later chapters. The use of these experiments will obviate the need for so many lessons in order to have the pupils acquire the subject matter.

In college work there is an excellent opportunity for making use of the greater number of the experiments cited later. Indeed, many additional variations may be used provided the necessary equipment is at hand.

The experimental work which is done in the lower grades will, of necessity, have to be closely supervised by the teacher. She should enlist the cooperation of all of the pupils in caring for the animals so that they will feel a personal interest in the project. Needless to say, it would be decidedly unwise to allow them to handle the animals at will, especially when they are on test. In high school even more of the responsibility for the experiments can be placed upon the students and in college work there should be no hesitancy at allowing them full control over the experiments. This does not mean in the latter case that full directions are not to be outlined. Students who have had no experience in handling test animals should be given the proper precautions. After their introduction to this work they should tend the animals in every respect, weighing, feeding, watering and making the other routine inspections.

Some students will exhibit a repugnance to the idea of working with rats, thinking them to be lowly animals associated with vermin—but not these animals. The highly bred Albino rat of today is kept in ideal surroundings—cage born and carefully dieted —and if attended properly will be a most desirable laboratory subject. Scientists are eager to pay homage to this servant of the human race. Thousands of lives have either been saved completely or made much more useful by virtue of rat experimentation. Preliminary evidence indicates that experiments now in progress have extended the prime of life in rats by dietary means. These results will doubtless be interpreted soon in terms of longer human life. Is it possible that such a beneficent animal as the white rat can be looked upon with a feeling of dislike?

Chapter II

THE CARE OF WHITE RATS

Upon first thought it would seem that the directions for the general care of white rats would be essentially the same regardless of the specific use for which they are maintained. The fact is that the directions for maintaining rats intended for tests could be followed (and are in commercial laboratories) even in the case of animals used for breeding or general utility purposes. However, since we are endeavoring to help the inexperienced teacher—inexperienced in the matter of caring for living rats—it seems wise to subdivide our instructions on care into two headings: (1) For Breeding and General Utility Purposes, and (2) For Test Purposes.

PART I

For Breeding and General Utility Purposes

In the small laboratory it is but seldom desirable to maintain a large colony of rats. The large group requires considerable space, more at least than is usually available when all of the other essential biological equipment is displayed to an advantage. Care is also a matter of concern—not difficult at all on small groups but in large colonies it is recognized as consuming much time if properly done. Therefore, for large groups of animals special adaptations and modifications will have to be made to our basic suggestions given here with particular regard for the smaller groups.

Cages

If one pair of rats is concerned, they may well be kept in a "feeding" cage, either a round or square type. It should be constructed of galvanized wire, preferably of $\frac{3}{8}''$ mesh for top and the sides. The bottom may well be made of $\frac{1}{2}''$ mesh as this size will allow the feces to drop through more readily. In a feeding experiment where an animal is on a deficient diet, it seems to sense the lack of some element from its diet and tries in every way to make up for it. This leads to the eating of its own feces if present in the cage and this would seriously interfere with the experiment. It is for this reason that the somewhat larger mesh is more satisfactory for this particular type of work. On the other hand, the larger mesh offers one disadvantage if the cage is used for breeding purposes in that the young rats can fall through the openings or get caught in them with disastrous results ensuing. This can largely be overcome by supplying the mother rat a liberal amount of shredded newspapers with which to build her nest. If this is done, and it is quite proper to do so, the young will remain in the nest until they are large enough to crawl about and then there is no danger of their falling through the mesh Some workers have a circular mat of $\frac{3}{8}''$ galvanized wire cut to fit into the cage over the regular $\frac{1}{2}''$ mesh bottom and this prevents any worry about the young.

The dimensions of the small round "feeding" cage (or, as we are describing it here —utility cage) can be approximately 10 inches in diameter and 10 inches in height. A slight variation either way is permissible. If the square type is preferred—and there seems to be no particular advantage to it except that the rats do seem to get a certain amount of satisfaction out of resting in the corners—then 10 inches to the side would give a cage with ample space for a pair of rats. In either case, the top should be removable or hinged to facilitate removing the animals for cleaning and this is a highly desirable feature for the bottom as well. An enamelware pan should be placed underneath the cage to catch the urine and fecal matter. There should be a distance of about one inch between the floor of the cage and the pan, this being accomplished by having a rim around the outside which will hold the floor up for this distance. If the cage receives daily attention, it can rest upon several thicknesses of newspapers but the pan will work out more practically in the long-run.

NUTRITION EXPERIMENTS

There are many types of rat cages on the market which answer various purposes admirably. But, many teachers wish to have their students take part in the construction of those cages to be used in their experiments. This work would be hard to justify on the basis of improving the students' skill in the manipulation of tools, for it would fall logically within the province of the manual training or machine shop course. It is justified, and amply so, when it serves to stimulate an interest in the project—where the mere mechanical work gives the student the desire to see the next step in the unfolding of the unit. The "hardware cloth" or galvanized wire screening can be purchased at almost any hardware store as can also the bolts, hinges and other accessories that one might wish to use for a cage of his own design. An intermediate step has been taken now by there being supplied "kits" or units of all of the materials required for a cage, disassembled, but ready to be put together, including the wire for the circular sides, formed tops and bottoms, hinge, rivets, screws and the various and sundry other small items that would go into a completed cage. (See page 38.) There are very obvious advantages in starting with this "semi-prepared" group of materials. The time for assembly will be greatly lessened, a much neater and more durable cage will result and the stimulation that comes to the student will be almost as much as though he had started from the rough materials purchased from the hardware store.

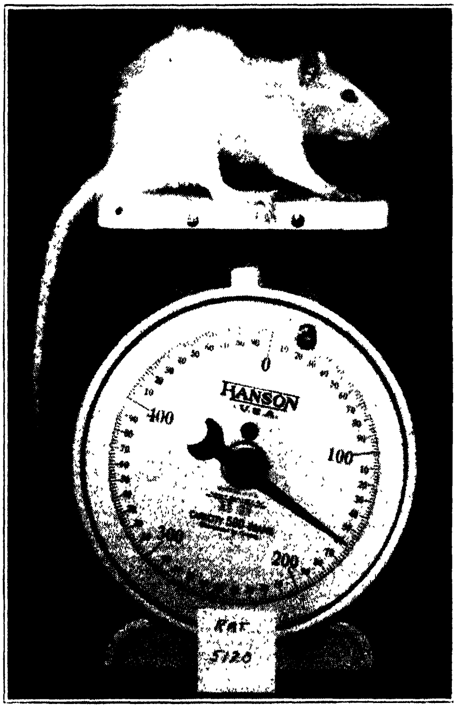
This question is likely to be raised at this point: "Why would not wooden cages be satisfactory?" Many express the belief that the rats appear to be more at home in the wooden cage—that the all-wire cage seems unnatural. The location of the cage is perhaps the factor which would most likely induce an uneasy feeling in the animal rather than the material out of which the cage might be constructed. Govern the other factors so essential to a happy rat colony and the cages will make very little difference in their state of contentment. There are so many points in favor of the wire construction which will be brought out in other connections that it must be given preference where serious study is attempted.

If several animals are to be kept in one cage, it must be of a larger size than that described above. It could be called a breeding or group cage. Like the smaller feeding cage, it should be made of galvanized wire of 3/8" mesh. The rectangular type is preferable in this instance and a convenient size is one 19 inches long, 12 inches wide and 9 inches high. The floor may likewise be of 3/8" mesh and the cage should rest in a rectangular galvanized pan of suitable size. The top should be hinged or there should be a special large door made in one side.

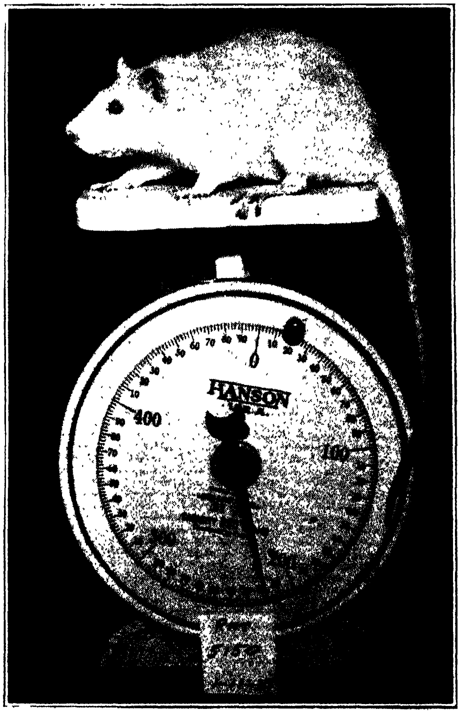
The cage should not be placed in a drafty place nor where direct sunlight will fall upon it. Rats should receive their sunlight only in the form of diffused radiation. The air in the room should be kept fresh and a temperature of 68° to 73° will be adequate. Sudden changes in temperature are harmful and should be guarded against as much as possible. Some arrangement must be made for keeping the animals warm in the schoolroom at night and on week-ends during the winter months.

There are only three absolute accessories for any rat cage used in this introductory work: a food or diet cup, a water fountain, and a data card holder with card record. By far the most satisfactory food receptacle is a small metal cup into which fits a metal collar having one small hole in it. The hole is just large enough to admit the rat's head and thus while feeding it is unable to scatter its food about. Keep in mind that the diet to be used will be dry powder and there is a tremendous waste if this is put out to the animals in a shallow open cup. There should, preferably, be some way of attaching the cup to the inside of the cage lest the older rats drag it about. The open water receptacle is out of the question in vitamin work and it is expedient that we eliminate it altogether from consideration here. The glass water fountain described on page 38 is satisfactory for all purposes, is inexpensive, and the one recommended instead of open glass saucers or cups. One can construct a glass bottle fountain in a few minutes by using either a test tube or small round bottle, a one-hole rubber stopper and a short piece (about 3") of glass tubing. One end of the tubing is sealed in a flame. Continue to apply heat to it uniformly and then blow through the other end so as to produce a bulbous enlargement in the heated portion. This will soon burst, leaving a small opening which is flamed. Heat the tube about one inch away from the enlargement and allow it to bend slightly. Insert the glass tube thus prepared into a one-hole rubber stopper which is then inserted into either a small bottle (2-ounce size is good) or vial. The filled fountain is inverted and attached to the outside of the cage, being careful that the glass tip protrudes only slightly through the open mesh into the interior. It should be at a convenient height for the animal.

The card holder is made of stiff metal for the back and a transparent front cover. There is a small hook at the top for fastening it directly to the cage. The data card (3″ x 5″) fits into the holder with ease, is always available and careful daily records are thus more easily kept.



Malnutrition due to poor protein content in diet and nutritional inbalance. Note humped back—evidence of poor calcium assimilation.



Same specimen as rat to left—after about 30 days on a complete diet. Note gain in weight, normal back curvature and generally healthful appearance.

Food and Water

Food and water are of primary importance in the growth and well being of any animals intended for breeding purposes. Particular attention is called to the fact that breeding rats are to have all of the important growth producing elements in the proper amounts. It has been said and truly so that a rat will eat almost anything providing it is starved to it—it will eat its own feces, a dead mate, paper, and even consumes enough metal from wire cages to throw off some of the exacting research experiments on anemia! But it does not follow that it prefers the improper foods.

NUTRITION EXPERIMENTS

Because the rat is an omnivorous creature some are tempted to feed it upon a few table scraps and let it go at that. It might live and get along fairly well but it will be more conducive to a good healthy body if a carefully compounded balanced ration is fed. A completely satisfactory stock diet is made up as follows:

Whole wheat flour	21 parts
Yellow corn meal	21 parts
Whole oat flour	20 parts
Whole milk powder	20 parts
Linseed oil meal	10 parts
Casein	3 parts
Yeast	3 parts
Calcium carbonate	1 part
Sodium chloride	1 part

This formula is easily compounded even by an inexperienced person the only requirement is that it should be mixed thoroughly. (See Chapter V for details on mixing diets.) The resulting mixture is a dry powder and it is to be fed this way. Over and over the question has arisen in regard to feeding a dry powder—many think it will not be eaten at all; others that the animal will not thrive upon it. All of the required elements are in this dry mixture and the rat will eat it. Take an old rat, accustomed to table scraps, vegetables, etc., and put it upon a dry powder diet and of course it will not eat it with relish at first. As it becomes hungry it will adapt its conception of good food to that which is available.

It is possible to supplement the stock ration (formula given above) with carrots, lettuce and perhaps other leafy vegetables, but this practice is not advisable as it tends to unbalance the ration. More latitude is permissible in feeding the breeding and utility stock than there is with the animals used in feeding experiments. One would wish however to give only the best of foods and test the advisability of using a certain food by asking yourself the question: "Is it fit for human consumption?" If not, don't try it on the rats.

The ingredients for mixing the stock diet can be purchased separately, or the already compounded diet can be bought if one does not care for the trouble of mixing it. Regardless of the form in which it is purchased, there should be kept on hand at all times several pounds of the food. Keep it well packaged and in a relatively cool dry place. It will not deteriorate if these precautions are observed.

The stomach of the rat is so small that it can not take in enough water to last it for a long time. Therefore, an abundant and constantly available water supply must be arranged. The glass water fountain already described satisfies these requirements. Either distilled or tap water may be used, provided the latter is suitable for human consumption. If not, it should be boiled.

Handling the Animals

The primary requisite in beginning a colony of rats, even though it be only an initial pair, is to be sure of the source from which they are obtained. There are "alley rats" as well as highly bred cage grown rats. By the former, we mean that sometimes the rats are reared in large enclosures, receive no individual care, are subject to the ravages of all rat diseases and they will not be at all suitable to the work outlined here. A case was reported recently where a pupil brought a pair of these rats to his teacher who wanted to use them in the biology course. Another boy was tending them and one of the animals rushed at his hand and cut a deep incision in one of his fingers. The board of health in the city impounded the rats for observation and began the Pasteur treatments on the boy. All ended well in this case but all people concerned were seriously inconvenienced for several days. All of this need not discourage anyone from the use of suitable rats but most surely it should warn those who contemplate using animals from an unknown source.

THE CARE OF WHITE RATS

A rat is as full of curiosity as a young child, always "nosing" around, finding out things. They are interesting to observe when given their freedom upon a table or when allowed to crawl up one's arm. They enjoy being petted and soon come to know their regular keepers. Starting with cage bred, young animals one will have no difficulty in gentling them. Stroke them gently a few times when they are fed or whenever it is necessary to place the hand in the cage. Talk to them, whistle to them and they will soon recognize your friendly intentions even before you stroke them.

One may choose his own technique in picking up a rat. Two good alternatives are used and one may adopt either one that comes natural to him: (1) take hold at the middle of the tail and allow the animal to rest its feet upon the palm of the other hand and (2) pass the index finger under the neck, just in front of the fore limbs and the other fingers about the belly between the fore and hind limbs—the thumb around the back of the neck. Always refrain from rapid or jerky movements as this will prevent the animal from becoming excited or frightened. Let your actions be positive but very deliberate. Carefully gentled animals will seldom if ever attempt to bite unless there is a provocation. Never place the hands in the cage unless they are clean and dry. A strong food odor on the hands may cause the rat to nip a finger.

In the event of any bite or scratch, sterilize the wound thoroughly, don't take anything for granted. Use 70% alcohol if available or tincture of iodine.

Cleaning the Cages

The cages should at all times be kept in a state of immaculate cleanliness. Coarse mesh wire for the bottom is decidedly advantageous from the sanitary standpoint. Very little refuse material will actually cling to the wires, most of it passing directly to the pan beneath. Should any feces adhere to the bottom, brush it through, empty and wash the pan daily. If there is any odor to the cage wash it in hot soap suds and rinse in clear hot water. Carefully dry it before replacing the animals in it. This will likely have to be done only twice a week. It is highly advisable to disinfect all cages and equipment at least weekly. These suggestions should eliminate any apprehension about odor—there will not be any as long as cleanliness is practiced.

If there is any contamination of the water supply, brought about by food particles having gotten to it by way of the rat's mouth, the container should be carefully washed and refilled with a fresh supply. Do not allow the animals to have access to a polluted saucer of water.

Breeding

The female rat matures sexually at about eighty days but it is far better not to allow her to breed for approximately another 20 days. Her litters will be larger and the young will be of better size. Sometimes there are only two or three pups in the first litter if the mother is young but usually the succeeding litters average about seven or eight young. The oestrous cycle of the rat is approximately four days, subject to variation up to seven or eight days. The gestation period is twenty-one or twenty-two days, subject of course to some variation.

Females will usually mate immediately after casting a litter if the male is present. It is wise to let the female rest for a period of from three to four weeks before allowing her to breed a second time. This gives her a chance to build up her own body reserves and thus to give better nurture to the next litter. When ready to mate again, place the male in the cage with the female and keep them together until the female shows signs of pregnancy. The surest sign is the abdominal swelling, particularly noticeable a few days before parturition occurs. The male should then be removed to another cage. His presence is disturbing to the female and this may cause her to kill the litter. The male would do no intentional harm to the young but it is best for him to be kept in a separate cage.

NUTRITION EXPERIMENTS

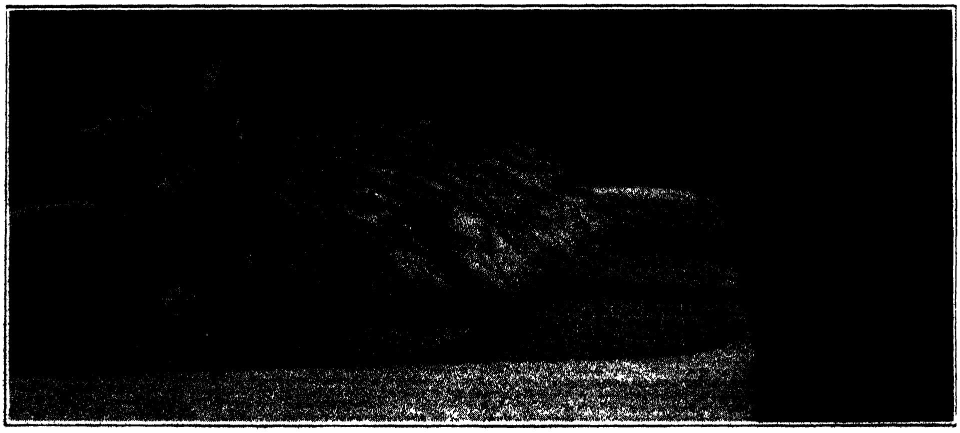
Provide the pregnant female with an abundant lot of newspaper shreds with which to build her nest. The nesting paper must be kept dry for wet paper will kill off a young litter within twenty-four hours. With gentled rats, it is possible to go ahead with daily handling during this period, but with those not so tame it is an entirely different matter. She will probably resent the intrusion and attempt to bite. Should even gentled stock become excited at this time, put forth an extra amount of talking and "singing" to them and often their fears can be completely dispelled through your own friendly attitude. It is entirely possible in the case of well gentled females to remove them from their young ones for weighing and general observations. This must be done cautiously for the first few times, however. Do not keep the mother away from the pups for more than a few minutes at a time.

The young are quite immature at birth—their eyes are completely closed and remain so for some fifteen days. At the time the eyes open one can detect size differences and any weaklings should be discarded. They will not develop into first-class animals. When 23 days old the young may be weaned although it does no harm to allow them to remain with the mother for a short time unless she is about ready to cast another litter. They will have been eating some food prior to this time so that weaning is not severe on them. The same stock diet as that given the mother will be relished by the youngsters. The young rats may be left together until they approach an age of eight weeks when the sexes should be separated lest breeding occur at this young age.

Anyone working with young animals needs to know how to distinguish between the sexes at an early age. The most reliable criterion is the distance between the anus and the genital papilla—it being greater in the male than in the female of equal age. In mature females the vaginal opening can be seen just posterior to the genital papilla.

The two sexes start out at pretty much the same weight at birth. There is a discernible difference at forty days and after that the male gradually increases in weight over the female until at seventy days of age there is a difference of about 13 grams. At this age the male weighs approximately 150 grams. (See chart on page 34.)

Rats normally live to be about three years old. Their most vigorous reproductive period is from three months on up to the end of the first year. The female will continue to breed after this period but the litters become small and intermittent. Commercially, it is unwise to keep and breed the worn out stock, hence the practice of eliminating them from the breeding colony when their prime has been exceeded.



Young rat about 30 days of age. Excellent illustration of the effect of poor lactation on the part of the mother.

Chapter III

THE CARE OF WHITE RATS FOR TEST PURPOSES

For any of the experiments mentioned later (except No. 3 of Chapter VII) it is preferable to use four young rats of identically the same age. Two are to be kept in one cage as the control group and there may be a male and a female in the group. If so, then, another male and female should be kept in another cage and to them the deficient diet will be fed. We may call the latter the test group. If one does not wish to use so many animals then one rat to the cage can be used provided they are of the same age and sex. The same sex is important as males and females gain at slightly different rates and we want an absolute comparison between the animals so as to test the effect of the food or the lack of a constituent, as the case may be—weight being an important criterion.

Cages

The small wire feeding cage described on page 5 is rather near the ideal type. The ½" mesh wire must be used for the bottom so that the feces will drop through readily. The diet cup and water fountain should be of the standard type recommended in the earlier pages. It need hardly be said except for emphasis that as near as possible the control and the experimental cages should be of the same size. This will admit of just as much exercise for the one group as for the other. We must remember that for the sake of accurate results the two groups of rats must be subjected to identical conditions.

Especial care must be exercised in keeping the cage and all accessories in a perfect state of cleanliness. Daily attention, except perhaps over the week-end, is none too often for such a matter as washing the cage pans and water fountains.

Food and Water

The food for the experiments will invariably be a dry mixture, not differing greatly in form from ordinary flour or starch. It will seem unnatural to feed this at first, but have no hesitancy if your stock has come from a large scale breeder, for the young 25-28 day old rats will have been started on a dry stock diet. Even though they have been reared in your own laboratory on a highly varied diet put them on the prescribed dry mixture at the appointed time. A short time may possibly elapse before it can be said that they enjoy this form of food but they will soon become accustomed to it. Feed this diet and this alone!

The diets that have been worked out for our experiments are the same for the controls as for the test animals, or at least basically the same, except for the particular food substance which is in question. The substance is present in the diet given to the control animals and absent from the diet given to the test rats. All other factors in the care being identically the same between the two groups, any signs of a less-than-perfect state of health in the test group which do not show up in the controls, must be attributed to the lack of that particular substance from their diet. We must re-emphasize this point, for time and again we have received orders for the deficient diet mixture alone to be used for the test animals and at the same time the persons have inquired whether it would be all right to use an entirely different food (perhaps a special dog food) for the control animals. An experiment using the two widely varying diets such as indicated here would be meaningless. Still others inquire whether some succulent vegetable should not be used to supplement the dry diet. This would most surely defeat the purpose of the experiment. One who loves the animals will deeply sympathize with the deficient animals when their symptoms appear but he must realize that this is the result we have been working for and that soon, after all the students have agreed that they have had this lesson on nutrition demonstrated in a most striking and concrete way, the animal can eat its way back to a happy state of health by way of a complete diet—the identical diet used for the control cage.

NUTRITION EXPERIMENTS

There is a classic example in one of our large universities that should be related for the lesson it will teach us about the utter precision with which we must apply our control over the diets of animals on test. A large group of rats was on a food test and after the normal length of time had elapsed for the appearance of deficiency symptoms in the test rats they were found to be in just as perfect a state of health as the animals in the control cage. A careful recheck of all possible sources for error was made but no clue was found. Finally, it was noted that the janitor enjoyed eating his lunch in the rat room and that out of a kind and sympathetic feeling toward this one cage of young pets he had been sharing his lettuce, meat and cheese with them! Moral—kind-hearted janitors will bear watching when working in the rat room.

The advantage in using the glass water fountain is that it will deliver water to the animal's mouth directly and there is little possibility of there being an excess adhering to the lips and hair which will then be taken to the food cup. If an appreciable amount were to get into the food then it would cause a production of microscopic flora in a short while and they in turn would tend to interfere with the experiment. The reverse can happen—food can be taken to the water fountain if the tip of the delivery tube is large enough and bent upward at an acute angle. Thus it is expedient to observe closely the water and food cups at short intervals.

Weighing Test Animals

Refer to page 14. Note that cage has been brought to the scale, animal is at ease, instant weight recording on a gram scale and especially that the entry is being made in the record sheet.

RAT HEALTH SIGNS

Chapter IV

RAT HEALTH SIGNS

Alertness or inquisitiveness.

Activity, especially in the evening.

Steady gait as contrasted with wobbly.

Regular respiration.

Absence of mucous secretion from nostrils.

Firm stools.

Absence of discoloration about anus and external genitals.

Sleek, slightly ivory colored hair, forming a smooth (not fluffy) coat over the body.

Complete absence of snuffles (occasional snuffles due to food may occur in perfectly healthy animals).

Plump, well rounded, and firm body.

Firm bones and normal teeth.

NUTRITION EXPERIMENTS

Chapter V

PREPARING FOR THE EXPERIMENTS

Mixing Diets

When several food experiments are planned, each using as a minimum four young rats, it is somewhat more economical to purchase the separate ingredients called for and mix them in your own laboratory. (Refer to page 37 for listings of the commonly used diet components.) This work can be done profitably by the students as they will be anxious to see just what actually goes into the diet mixture. In cases where only one feeding experiment is to be conducted, time can be saved by obtaining the proper amount of the already mixed diet.

Following out the same idea as that mentioned in connection with the "semi-prepared" cages, it has now been made an easy matter to purchase a set of unmixed ingredients, each in the proper amount for blending into enough of the diet to take the animals through the experiment. (See page 35 for "Unmixed diet components sets.") A very few substances which deteriorate if kept at room temperatures, such as butter, lard and Cod liver oil, should be purchased locally and for this reason are not included in the sets. Viosterol is sold in small units (5 cc) and it is more practical to purchase it in this size of bottle rather than try to obtain just the few drops at one time that are required for one certain diet.

After the various ingredients have been weighed out in their correct percentages, (the Hanson platform scale listed with the other supplies is most efficient for this), they are ready for mixing. A large sheet of clean paper (size of a newspaper—or a newspaper can be made to serve the purpose) is spread out on the table. The carbohydrate component is emptied upon the paper, it being best to start with it especially when fats even in small quantities are to be included in the same diet. Next add the fat, be it butter, lard or Cod liver oil, and with clean fingers begin working it into the carbohydrate component. Next add the protein component and thoroughly work the mixture. Salts or any other ingredients can now be added. Work the whole for at least ten or fifteen minutes and a homogeneous mixture will result. The appearance and the feel of it will be sufficient indication as to when the mixing may cease.

Two rats, twenty-eight days of age at the beginning of an experiment, will consume between 2½ and 3 pounds of the diet by the end of their first month on test. It is well to mix up at least a one-month's supply of the food at one time. The foods will usually keep very well if they remain in a reasonably cool dry place. Vitamin D deficient diets, for obvious reasons, should never be placed where sunlight will reach them.

Weighing

Both test and control animals should be weighed daily. By beginning this procedure while they are still small they will grow up with this as merely a part of their expectable routine. If the animal when first removed from the cage seems nervous and excitable there is but little chance that it will stand upon the scale for even the brief moment required for noting the exact weight. Pet it gently with the free hand while holding it in the palm of the other and it will quiet down readily. It takes only a few times to overcome any fear that the rat has of the weighing process. The same person should weigh the animals each time. Changing attendants for any of the work with the rats is advised against if best results are expected.

EXPERIMENTS

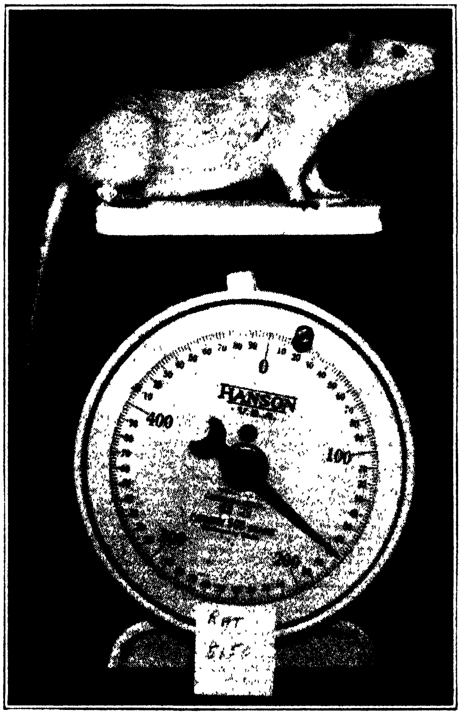
Chapter VI

EXPERIMENTS UPON CLASSES OF FOOD SUBSTANCES

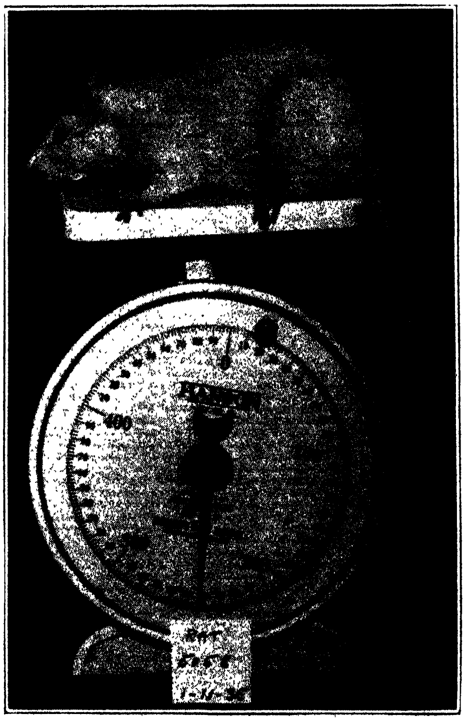
(1) Demonstration of the Value of Protein

Proteins are the muscle builders of the food family. Cells and tissues of the body are being destroyed constantly and are only replaced when an adequate supply of protein is eaten. There is a definite distinction between plant and animal protein and the human body cannot live without meat or animal protein in some form, because it is not able to satisfactorily make use of plant proteins to build tissues.

The protein necessary for the healthy body is obtained from meats (eggs and fish included) and milk. Plant or vegetable protein is useful as a supplement to meat proteins, but is not absolutely necessary. As will be seen later, however, vegetables are very necessary for the building of a healthy body.



Malnutrition due to protein deficiency.



Same animal after a 20-day recovery period on a protein sufficient diet.

Experiment

EQUIPMENT

2 cages with food cups and water tubes.
3 pounds of the deficient diet.
3 pounds of the sufficient (control) diet.
1 scale graduated in grams.

NUTRITION EXPERIMENTS

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. It is extremely important that these animals come from a reliable and guaranteed stock.

CARE

Each group is housed in a separate cage. To one group feed a protein-free diet as outlined below, the other being fed the same diet except that a sufficient source of protein (milk) is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records should be kept on all animals.

Protein Deficient Diet Group

The first group is placed on a protein deficient diet as follows:

Corn Starch	92 parts
Lard	3 parts
Sodium Chloride	1 part
Calcium Carbonate	2 parts
Yeast	2 parts

Cod liver oil, 40 drops per one thousand grams of diet.

This diet has the necessary fat, carbohydrates, minerals and vitamins, but is lacking in adequate protein.

Complete Diet Group

The second group is placed on the following diet which will be complete.

Milk	32 parts
Corn Starch	60 parts
Lard	3 parts
Sodium Chloride	1 part
Calcium Carbonate	2 parts
Yeast	2 parts

Cod liver oil, 40 drops per one thousand grams of diet.

Results

The difference between the two groups should become evident almost immediately. In the first group the animals will not gain weight and will remain small, the second group growing normally. After about 15 days the first group will start losing their hair and probably be practically unable to walk. There will be no doubt at this point of the necessity for eating protein.

Cure of Deficient Animals

It may be desirable to show the recovery of these animals when placed upon a complete diet. This may be done by changing the diet of the deficient group after they have become quite poor to that of the control group. Evidences of recovery will be present almost immediately and if continued in this way for a sufficient period of time the animals will return to normal health.

(2) Demonstration of the Value of Carbohydrates

The carbohydrate part of the diet is the factor we all depend upon primarily to give us energy. Without any source of carbohydrate material animals and humans both, would soon die. Carbohydrates also play a more subtle role in the diet, namely that of aiding in the digestion of protein material. This fact is overlooked by a great number of people, particularly those who advocate the ingestion of carbohydrate material and protein material separately. There are, of course, certain types of carbohydrates that are nondigestible. These certain ones serve the purpose of keeping the intestine cleaned and active and prevent a condition of diarrhea. The demonstration of a lack of carbohydrates is very simple and quite quickly shown. We shall not attempt, however, in this booklet, to give a demonstration of a lack of nondigestible carbohydrates.

The chief sources of carbohydrates are: breads of all kinds, potatoes, milk, sugar and root vegetables. Sources of the valuable nondigestible carbohydrates are leafy vegetables such as spinach, cabbage, lettuce, etc.

Experiment

EQUIPMENT

2 cages with food cups and water tubes.
3 pounds of carbohydrate deficient diet.
3 pounds of carbohydrate sufficient diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. It is important that these animals come from a reliable and guaranteed stock.

CARE

The animals are housed in separate cages but one cage for each group will suffice. One group will be fed a carbohydrate free diet as outlined below, the other being fed the same diet except that a sufficient source (corn starch) of carbohydrate is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records should be kept on all animals.

Carbohydrate Deficient Diet Group

The first group is placed on a carbohydrate deficient diet having this formula:

Casein	35 parts
Cellu-flour	55 parts
Butter	5 parts
Sodium Chloride	1 part
Calcium Carbonate	2 parts
Yeast	2 parts

Cod liver oil, 40 drops per one thousand grams of diet.

This diet has the necessary proteins, fat, minerals and vitamins but is lacking in an adequate carbohydrate. The cellu-flour is a nondigestible carbohydrate.

Complete Diet Group

The second group is placed on the following diet which will be complete.

Casein	30 parts
Corn Starch	60 parts
Butter	5 parts
Sodium Chloride	1 part
Calcium Carbonate	2 parts
Yeast	2 parts

Cod liver oil, 40 drops per one thousand grams of diet.

Results

The difference between the two groups should be noticeable almost immediately. The animals on the deficient diet will probably not live for more than 25 days. The deficient group will be particularly inactive as compared with the controls and will show practically no gain in weight. The more active the animals are kept during the day, the more marked the difference in gains between the two groups, due to the fact that the control group is receiving, per gram, considerably more energy. From 10 or 15 days after the beginning of the demonstration there should be no doubt about the difference between the two groups of animals and care must be taken to prevent the deficient group from dying.

Cure of Deficient Animals

As soon as the rats of the first group show a marked difference, when compared, it may be desirable to show the recovery of the animals. This may be done by changing the diet of the deficient group to that of the control group.

(3) Demonstration of the Value of Fats

Small quantities of fat deliver very large amounts of energy to the body. Fats are extremely valuable to persons who are exercising or exerting themselves to a very great extent. It is true, of course, that carbohydrates are responsible for a very great part of the energy-giving material delivered from food, but the fact that such small amounts of fat will make up for a comparatively large amount of carbohydrate makes them exceedingly valuable. It is sometimes quite difficult to demonstrate the effect of a lack of fat in the diet. However, with care and patience this can be demonstrated nicely.

Fat in the diet is obtained from products such as milk, butter, cheese, and to a lesser degree from meats, particularly if they are cooked in some fat such as lard.

Experiment

EQUIPMENT

2 cages with food cups and water tubes.
3 pounds of fat-free diet.
3 pounds of the fat-complete diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. The animals for this experiment should be laboratory grown as much better results will follow if standard specimens are used.

CARE

The animals are housed in separate cages but one cage for each group will suffice. One group will be fed a fat-free diet as outlined below, the other being fed the same diet except that a sufficient source of fat (butter) is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records are especially important in this experiment.

Fat Deficient Diet Group

The first group is placed on a fat deficient diet as follows:

Casein	40 parts
Corn Starch	55 parts
Sodium Chloride	1 part

Calcium Carbonate	2 parts
Yeast	2 parts
Cod liver oil, 40 drops per one thousand grams of diet.	

This diet has the necessary protein, carbohydrates, minerals and vitamins but is lacking in adequate fat.

Complete Diet Group

The second group is placed on the following diet which will be complete.

Casein	30 parts
Corn Starch	60 parts
Butter	5 parts
Sodium Chloride	1 part
Calcium Carbonate	2 parts
Yeast	2 parts
Cod liver oil, 40 drops per one thousand grams of diet.	

Results

The difference between the two groups will not be nearly so marked as in the case of the protein deficiency and probably will not be evident before the end of 30 days. At the end of 30 days the animals on the fat deficiency group should be less active than the other group and should not show the rate of gain that the second group shows. It is very possible that the animals on the deficient diet will show a loss of hair and the hair itself will not be as white and nice as the hair of the animals in the second group. The more active the animals are kept during the day the more marked will be the difference in gains between the two groups, due to the fact that the second group is receiving a diet that delivers, per gram, considerably more energy.

Cure of Deficient Animals

If the rats of the first group show a marked difference, when compared, it may be desirable to show the recovery of these animals when put on a complete diet. This may be done by changing the diet used for the test group to that which is used for the control animals.

(4) Demonstration of the Value of Minerals

Minerals play such an important part in the diets of all animals that it would be worth while to devote more space to that single study than almost any other.

Without an adequate supply of minerals the bones, tissue and blood suffer to a marked extent. A lack of calcium, for example, will cause the formation of poor, weak bones and will also profoundly effect the quality of the blood. A lack of phosphorus will cause a similar condition but not quite so marked as in the case of calcium. A lack of iron will produce anemia. A lack of iodine in the diet will permit enlargement of the thyroid gland, thereby producing a condition commonly known as goiter. A lack of copper in the diet will prevent the body from making use of any iron which it might receive and thereby produce anemia. Zinc, sulphur, sodium, potassium, chlorine, magnesium, manganese, all play equally important parts in the proper development and maintenance of the body. The study as outlined below will show the effect of a complete lack of minerals.

The best way to be certain of getting all these minerals is including milk and green or leafy vegetables, preferably in the raw state, in the diet.

NUTRITION EXPERIMENTS

Experiment

EQUIPMENT

2 cages with food cups and water tubes.
3 pounds of mineral deficient diet.
3 pounds of a mineral sufficient diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. We must point out that one animal can be used in each cage, instead of two, in which case, the same sex should be used. Two animals are far better because there might be individual variations not easily explicable if there is only one animal to the cage.

CARE

The animals are preferably housed in separate cages but one cage for each group will suffice. One group will be fed a mineral free diet as outlined below, the other being fed the same diet except that a sufficient source of mineral (Salts No. 51) is added. Food is kept before both groups of animals at all times. Water supply at all times must be adequate and should be inspected frequently to be certain it remains clean. Daily weight records should be kept on all animals.

Mineral Deficient Diet Group

The first group is placed on a mineral deficient diet as follows:

Casein	15 parts
Lard	4 parts
Corn Starch	74 parts
Agar Agar	6 parts
Yeast	1 part

Cod liver oil, 40 drops per one thousand grams of diet.

Complete Diet Group

The second group is placed on the following diet which will be complete.

Casein	14 parts
Lard	3 parts
Corn Starch	70 parts
Agar Agar	5 parts
Yeast	1.9 parts
Salts No. 51	6.1 parts

Cod liver oil, 40 drops per one thousand grams of diet.

Salts No. 51 is composed as follows:

Calcium Carbonate	1.5 parts
Potassium Chloride	1.0 parts
Sodium Chloride	0.5 parts
Sodium Bicarbonate	0.7 parts
Magnesium Oxide	0.2 parts
Ferric Citrate	0.5 parts
Monobasic Potassium Phosphate	1.7 parts

Results

The difference between the two groups should be obvious within 30 days. A cessation of growth is the first evidence of a lack of minerals. At about 25 days anemia will probably be evidenced by the paling of the ears, eyes and feet. The animals will have a poor coat of hair and will appear generally unhealthy. It is very probable that before the end of 30 days all the animals on the deficient diet will develop colds or a mild case of pneumonia.

Cure of Deficient Animals

At the end of 30 days it may be desirable to show the recovery of these animals when put on a complete diet. This may be done by changing the diet of the first group to that of the second and continuing the study for a period of about 30 more days.

Special Microscope Slides

to show
the difference between normal and rachitic bone.
(See page 26 Vitamin D)

H12.51	**Rachitic bone,** l. s. of the end of one of the limb bones showing degeneration due to rickets	$0.75
H12.52	**Normal rat bone,** l. s. for comparison with rachitic bone	.75

VITAMIN EXPERIMENTS

Chapter VII

QUALITATIVE VITAMIN EXPERIMENTS

(1) Demonstration of the Value of Vitamin A

As early as 1909 the existence of a factor that effected growth was recognized. It was also recognized that very minute quantities of this unknown material profoundly influenced the growth of a great number of different animals, including humans. Recently the chemical structure of this substance, known as vitamin A, has been discovered. A complete lack of vitamin A in the diet will result in complete lack of growth and eventual death. Accompanying this cessation of growth is an inflammation of the eyes known as xerophthalmia. The resistance of the body to disease of all kinds is lowered considerably and as a result the subject that is not receiving a source of vitamin A seldom dies directly from malnutrition but rather from diseases such as pneumonia or other bacterial infection.

The most valuable common sources of this vitamin are eggs, butter, milk, some cheeses, green leafy vegetables and some root vegetables, particularly carrots. A glance at this list of foods will suffice to show how easy it is to obtain a proper amount of vitamin A daily.

Experiment

EQUIPMENT

2 cages with food cups and water fountains.
3 pounds of vitamin A deficient diet.
3 pounds of vitamin A sufficient diet for the control group.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. In any of these experiments, more than two animals may be used, and perhaps better results will ensue. For a larger number there would have to be a corresponding increase in the amounts of each of the above diets.

CARE

The animals may be housed in separate cages but one cage for each group will suffice. One group will be fed a vitamin A free diet as outlined below, the other being fed the same diet except that a sufficient source of vitamin A (Cod liver oil) is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records should be kept on all animals.

Vitamin A Deficient Diet Group

The first group is placed on a vitamin A deficient diet as follows. This diet was outlined by Sherman in the Journal of Biological Chemistry 1930, 88, 683.

Casein	18 parts
Yeast	10 parts
Corn Starch	67 parts
Calcium Carbonate	3 parts
Sodium Chloride	2 parts

Irradiated commercial Cholesterol, 15 drops
per one thousand grams of diet.

This diet has the necessary protein, carbohydrates, fat, minerals and all vitamins except A.

Complete Diet Group

The second group is placed upon the following diet which is the same as the deficiency diet except a source of vitamin A is added.

Casein	18 parts
Yeast	10 parts
Corn Starch	67 parts
Calcium Carbonate	3 parts
Sodium Chloride	2 parts

Irradiated commercial Cholesterol, 15 drops per one thousand grams of diet.

Cod liver oil, 20 grams per one thousand grams of diet.

Results

The difference between the two groups should be obvious within 30 days. Cessation of growth will occur at about the eighteenth day. The condition of xerophthalmia will become evident between the 20th and 25th test day.

The eyes will become inflamed and swollen and may even become so severe as to shut entirely. The animals on the deficient diet will in all probability develop colds with slight nasal hemorrhages as evidenced by a bloody appearance about the nostrils. Particular care must be taken in keeping the animals in a sheltered, non-drafty place in order to minimize the chance of the development of severe pneumonia.

Cure of Deficient Animals

At the end of 30 days it may be desirable to show the recovery of these animals when put on a complete diet. This may be done by changing the diet of the first group to that of the second and continuing the study for a period of about 30 more days.

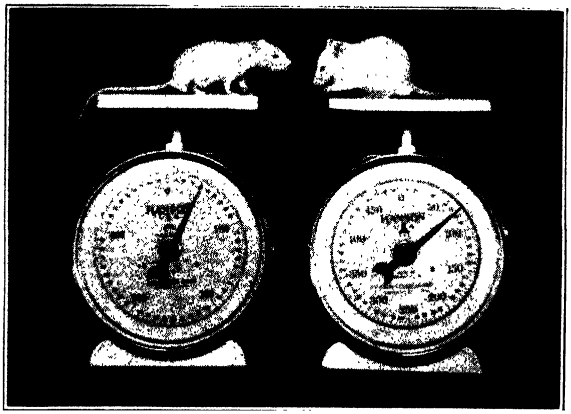
It is very possible to demonstrate the existence of vitamin A in various common foodstuffs and it may be desirable to do so. Follow the procedure as just outlined but instead of placing them on the complete diet as outlined, continue to feed them the deficient diet but give them milk to drink instead of water; or add a good grade of butter to the diet; or feed the animals a supplement of lettuce, carrot tops or spinach; or, fourthly give the animals a supplement of whole, fresh, uncooked carrots. The recovery on any or all of these plans will become evident almost immediately, provided the animals have not reached too complete a stage of depletion.

(2) Demonstration of the Value of Vitamin B (B_1)

It was not until more recently that the existence of the so-called Vitamin B was recognized. Animals fed a diet of casein, starch, butter fats, water, and salts fail to grow and subsequently develop polyneuritis. This effect takes place with humans as well as lower animals. Small amounts of yeast are found to be sufficient to correct this deficiency, but what is more important is that as little as 2 ounces of *milk* a day is sufficient to prevent polyneuritis. The lack of vitamin B has another interesting result, namely that of producing a lack of appetite. The effect of this is, of course, obvious.

There is a belief that brown bread is dietetically better than white bread, primarily because it contains more vitamins than white bread and particularly vitamin B. Both breads are made with yeast, which is the most potent source of vitamin B known. To quote Graham Lusk: "A number of people *assume* that brown bread has a far greater dietetic value than white bread, but there are very few experimental data to warrant such an assumption." The difference, which is very slight, lies in the protein content, *not* in the vitamin content.

VITAMIN EXPERIMENTS



35-day-old litter mates illustrate the effect of a lack of vitamin B in the diet. Rat on left weighs only 40 grams—normal or control on the right, 75 grams.

There are many sources of vitamin B, namely: yeast, milk, green vegetables, juices of fresh oranges, lemons and grapefruit, prunes, and liver. There should certainly be no worry about not receiving sufficient vitamin B in the diet because of the imposing array of foodstuffs containing it.

Experiment

EQUIPMENT

2 cages with food cups and water tubes.
3 pounds of vitamin B free diet.
3 pounds of vitamin B sufficient diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. They should be standard in all respects, having come from a reliable colony.

CARE

The animals are housed in separate cages but one cage for each group will suffice. One group will be fed a vitamin B free diet as outlined below, the other being fed the same diet except that a sufficient source of vitamin B (the dried yeast) is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records should be kept on all animals.

Vitamin B Deficient Diet Group

The first group is placed on a vitamin B deficient diet as follows. This diet is the one outlined by Evans and Lepkovsky in the Journal of Nutrition, 1929, 2, 1.

Casein	40 parts
Sucrose	45 parts
Salts No. 51	4 parts
Autoclaved yeast	10 parts
Cod Liver oil	1 part

This diet has the necessary proteins, carbohydrates, fats, minerals, and vitamins, except vitamin B.

Complete Diet Group

The second group is placed upon the following mixture which is the same as the deficiency diet except a source of vitamin B is added.

Casein	40 parts
Sucrose	45 parts
Salts No. 51	4 parts
Dried yeast	10 parts
Cod Liver oil	1 part

Results

The difference between the two groups should be obvious within 25 days. Cessation of growth will occur at about the 8th day. The condition of polyneuritis should become evident on about the 25th day. The animals will become weak and excitable, and a rigidity of the muscles will be obvious. The animals on the deficient diet will probably be almost unable to feed at the end of the 30 days' test period.

Cure of Deficient Animals

At the end of 30 days it may be desirable to attempt the recovery of these animals by placing them on a complete diet. This may be done by changing the diet of the first group to that of the second and continuing the study for about 30 more days.

It is very possible to demonstrate the existence of vitamin B in various common foodstuffs and it may be desirable to do so. Follow the procedure as just outlined but instead of placing them on the complete diet, continue to feed them the deficient diet but give them milk to drink instead of water; or feed the animals a supplement of lettuce, carrot tops or spinach. The recovery on any or all of these plans will become evident almost immediately, provided the animals have not reached too complete a stage of depletion.

(3) Demonstration of the Value of Vitamin C

Vitamin C, or rather the lack of it, has played an important part in history. Scurvy, caused by a vitamin C deficiency, is mentioned wherever records of sea journeys are found. Long before anything was known about vitamin C, or even that there were such things as vitamins, citrus fruit juices were used to combat scurvy. A steady loss of weight, with death occurring in a very short time is characteristic of scurvy. The teeth loosen, the gums are affected, and hemorrhages frequently occur in the skin and especially around the knee joints. Milled grains lack vitamin C, but unmilled grains contain it. If the daily diet contains milk, fresh vegetables and citrus fruit juices, there will be a great sufficiency of vitamin C.

Some mammals and fowls, notably rats, dogs, cows, chickens and pigeons manufacture vitamin C within their own bodies. For this reason the rat cannot be used to demonstrate vitamin C deficiency. Guinea pigs, however, give a definite scorbutic reaction in a short time.

Experiment

EQUIPMENT

2 cages with food cups and water fountains.
5 pounds of vitamin C deficient diet.
5 pounds of vitamin C sufficient diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of young guinea pigs weighing between 250-300 grams are needed. It is important that these animals come from a reliable stock.

CARE

The animals may be housed in separate cages but one cage for each group will suffice. One group will be fed a vitamin C free diet as outlined below, the other being fed the same diet except that a sufficient source of vitamin C (lemon juice) is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records should be kept on all of the guinea pigs.

Vitamin C Deficient Diet Group

The first group is placed on a vitamin C deficient diet as follows. This diet is from the method of Zilva as described in the Medical Research Council Special Report, No. 146, 1930.

Bran	38 parts
Barleymeal	12 parts
Middlings	20 parts
Fish Meal	6 parts
Crushed oats	24 parts

Complete Diet Group

The second group is placed upon the following diet which is the same as the deficiency diet with the exception that a source of vitamin C is added.

Bran	38 parts
Barley Meal	12 parts
Middlings	20 parts
Fish Meal	6 parts
Crushed oats	24 parts

Lemon juice, 10 c.c. for each 100 grams of diet.

Results

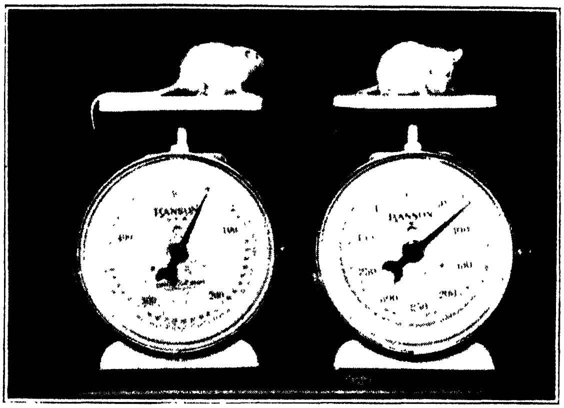
The animals on the scorbutic diet may gain weight for about two weeks, but after this period they will lose rapidly and die at the end of four or five weeks. The teeth loosen and the gums become congested with blood. Hemorrhages may appear in the skin, especially around the knee joints and the rib cartilages.

Cure of Deficient Animals

If it is desired to cure the scorbutic animals, they must be put on the vitamin C sufficient diet at the end of about three weeks. If the vitamin C value of any particular foodstuff is to be demonstrated continue feeding the scorbutic diet and add a special food, such as raw milk or potatoes. Other sources of the vitamin are: fresh vegetables, dried peas, unmilled grains, and tomato juice. The recovery should take a very short period if the animals are not too near death when the cure is begun.

(4) Demonstration of the Value of Vitamin D

The sight of a rachitic person is not as common as it once was, because much has been learned about the function and sources of vitamin D. Bow legs, knock knees and malformed chest and skull are the characteristics of rickets. Quite a few things, including lack of exercise, have been named as causative agents for rickets, but only within the last twenty years approximately has it been realized that diet was concerned. In rickets, calcium is not deposited in the bones and teeth. The bones are not strong enough to bear the weight of the individual and become malformed. An anti-rachitic diet must contain sufficient calcium and phosphorus *plus* vitamin D. All of the calcium and phosphorus in the world would be of no use to the individual if the vitamin D was not present to cause it to deposit in the bones and teeth.



The vitamin D deficiency shows up in the weight as well as in other marked characteristics. The test rat on the left weighs 41 grams; the control on the right 75 grams and they are litter mates.

Many of our foods which are rich in the other vitamins are not naturally a good source of vitamin D. Some of them may be made a good source by proper irradiation. Natural sources which are good are butterfat, egg-yolk, and animal and fish fats.

Vitamin D is the one vitamin which the human body can manufacture in small quantities and this only in the presence of direct sunlight or ultra-violet light. If fish oils are irradiated with either sunlight or ultra-violet light vitamin D is manufactured from the ergosterol and small doses of these special preparations are sufficient to prevent rickets. But it is better, as in the case of all of the other vitamins, to get the supply from foodstuffs.

Experiment

EQUIPMENT

2 cages with food cups and water tubes.
3 pounds of the vitamin D deficient diet.
(Note the deficient diet must not be kept where sunlight will fall upon it.)
3 pounds of the vitamin D sufficient diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. As is explained elsewhere, the age may vary slightly, that which is given is only preferable.

CARE

The animals are housed in separate cages but one cage for each group will suffice. One group will be fed the vitamin D free diet outlined below, the other being fed the same diet except that a sufficient source of vitamin D (Viosterol) is added. Both groups are fed their respective diets ad libitum. The animals must not receive *direct* sunlight and *preferably not any at all.* Water supply at all times must be adequate. Daily weight records should be kept on all animals.

Vitamin D Deficient Diet Group

The first group is placed on a vitamin D deficient diet as follows. This diet is the one suggested by Steenbock and Block in the Journal of Biological Chemistry, 1925, 64, 263.

Yellow Corn	76 parts
Gluten Flour	20 parts
Sodium Chloride	1 part

Calcium Carbonate	3 parts

This diet is rich in calcium and low in phosphorus and Vitamin D.

Complete Diet Group

The second group is placed upon the following diet which is the same as the deficiency diet with the exception that a source of Vitamin D is added.

Yellow Corn	76 parts
Gluten Flour	20 parts
Sodium Chloride	1 part
Calcium Carbonate	3 parts
Viosterol, 15 drops to one thousand grams of diet.	

Results

The animals on the rachitic diet will lose weight, and should show the effects after a period of three weeks or so, depending upon the age of the animal and its stored up supply of vitamin D at the beginning. The animal will develop a queer gait, walking on its toes and the legs will be bowed. Swelling of the knee joints may appear. The animal will grow in length for a while, but will be thin and eventually all growth will cease.

Cure of Deficient Animals

After the symptoms above have been noted the animals may be put on the vitamin D sufficient diet. If any particular foodstuff is to be tested, substitute it for the Viosterol. A larger quantity of the foodstuff will be necessary because the Viosterol supplies the vitamin D in a concentrated form.

It is said that an over-supply of vitamins does no harm, but along with this experiment it is possible to show that avitaminosis can be induced. To another group of animals feed the complete diet, but increase the Viosterol content several times. The animals will become very irritable, tremble and generally appear to be ill. Reducing the vitamin D supply again will cure them.

(5) Demonstration of the Value of Vitamin G (B_2)

Vitamin G, more often referred to as vitamin B_2 is a part of the vitamin B complex. There are several more factors in the complex about which little is known, and they will not be treated here.

Pellagra, caused by a deficiency of vitamin G, is a common disease in the United States. It is characterized by skin lesions which cause the skin to appear very dark, growth stops, the hair is lost, and it often causes death. Vitamin G is found in goodly quantities in yeast, liver, kidney, egg white, green cabbage and spinach, and in lesser amounts in most fruits, vegetables and dairy products.

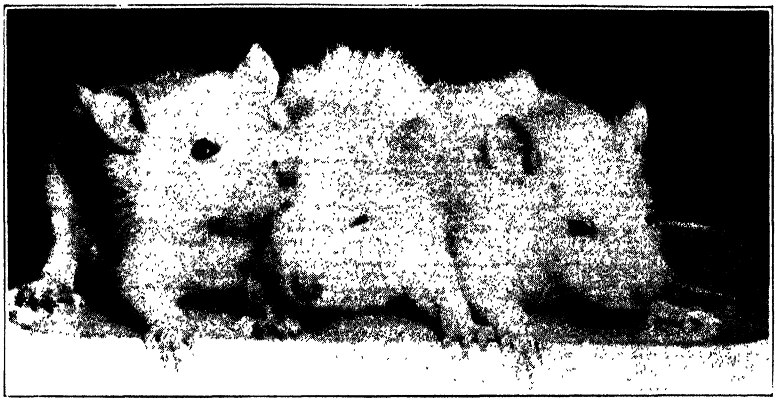
Experiment

EQUIPMENT

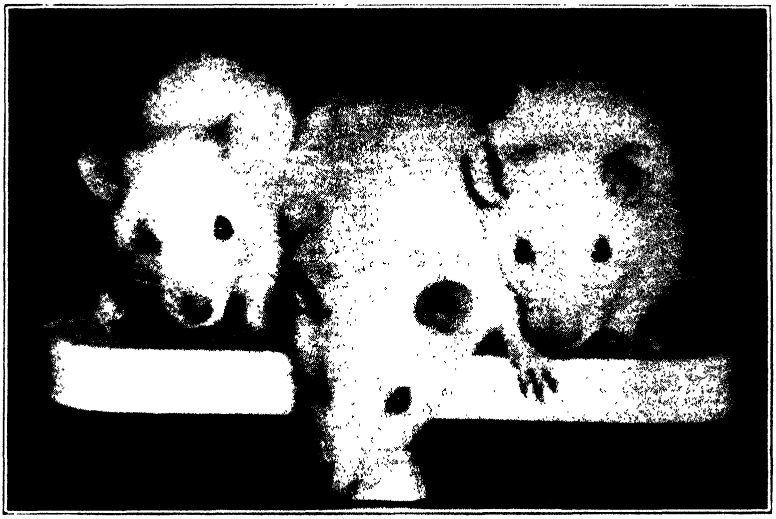
2 cages with food cups and water tubes or fountains.
3 pounds of the vitamin G deficient diet.
3 pounds of the vitamin G sufficient diet.
1 scale graduated in grams.

ANIMALS

Two groups (one male and female in each group) of standard 26 day old Albino rats all weighing between 45-50 grams are needed. It is extremely important that these animals come from a reliable and guaranteed stock.



Compare these little fellows that have been on diet deficient in vitamins B and G with their later picture below.



Same animals as those shown above after having received a diet to which vitamins B and G had been added.

CARE

The animals are housed in separate cages but one cage for each group will suffice. One group will be fed a vitamin G free diet as outlined below, the other being fed the same diet except that a sufficient source of vitamin G is added. Both groups are fed their respective diets ad libitum. Water supply at all times must be adequate. Daily weight records should be kept on all animals.

Vitamin G Deficient Diet Group

The first group is placed on a vitamin G deficient diet as follows:

Casein	18 parts
Starch	38 parts
White corn	30 parts
Lard	8 parts
Cod Liver oil	2 parts
Salts No. 51	4 parts

VITAMIN EXPERIMENTS

Complete Diet Group

The second group is placed upon the following diet which is the same as the deficiency diet with the exception that a source of vitamin G is added.

Casein	18 parts
Starch	38 parts
White corn	30 parts
Lard	8 parts
Cod Liver oil	2 parts
Salts No. 51	4 parts

Add 10% autoclaved yeast as a source of vitamin G.

Results

Growth should cease after a short period and there will be a loss of hair. Skin lesions and perhaps cataracts of the eyes will appear.

Cure of Deficient Animals

After these symptoms have been noted the animals should be put on the complete diet. One of the foodstuffs outlined above may be substituted for the autoclaved yeast if desired, but the yeast is the most potent supply of the vitamin G. In using natural foods to show the existence of this vitamin it will be necessary to feed larger proportional amounts than when feeding yeast.

In this booklet we have described experiments that will prove that a complete diet can be planned from natural foodstuffs and that concentrates are not necessary for health. Milk, as you will have noticed in the foregoing chapters, is mentioned as the source of many of the food elements. It is especially important in that the elements are in a very easily assimilated form. Although the vegetarian claims that his diet is complete, you will notice that meats supply not only protein, but certain minerals and vitamins. These particular minerals and vitamins are not found in conjunction with the vegetable proteins. Therefore it is essential that meat be included in the diet. Carbohydrates may be derived from various sources, but it is well to get them from several sources rather than just one. One vegetable, perhaps, may have a plentiful supply of one vitamin, but be lacking in another. By adding another vegetable, the vitamin supply can be made adequate. Another valuable property of most of the vegetables is that they supply roughage to keep the intestinal tract in a healthy condition. Enough fat will be taken in in conjunction with the meats and dairy products to meet the body needs, but as some of the vitamins are found only in fats, they are necessary also as a source of these vitamins.

The elements of the various foodstuffs, and the function of these elements in the body is too large a subject to be treated here. It is enough to say that if the daily diet contains fats, carbohydrates, minerals and proteins, from animal and vegetable sources supplemented by dairy products, *especially* milk, there need be no fear of the deficiency diseases.

Chapter VIII

QUANTITATIVE DETERMINATION OF VITAMIN A

Material to be Tested

Some type of commercial Cod liver oil will be most suitable for this test.

Animals

Twelve twenty-five day old Abino rats—mixed sexes permissible.

Equipment

Four cages.
Scissors for clipping ears.
One scale graduated in grams suitable for weighing animals.
One 1 cc hypodermic syringe with 20 gauge needle, filed to make a blunt end.
One hydrometer and jar for measuring the specific gravity of liquids lighter than water.
One food cup for each cage.
One drinking fountain for each cage.
One container suitable for storage of diet.

Vitamin A Deficient Diet

Purified Casein	18 parts
Salt Mixture No. 51	4 parts
Dried Brewer's Yeast	10 parts
Sodium Chloride	1 part
Corn Starch	67 parts

Irradiated commercial Cholesterol 25 drops per thousand grams of diet.

Procedure

Place three animals in each cage, notching the right ear of one, the left ear of another and allowing the ear of the third animal to remain intact. Keep all records according to cage numbers together with the ear mark. Feed the vitamin A deficient diet exclusively, together with an adequate supply of water ad libitum. Keep very accurate *daily* growth records. As soon as the growth curve shows a consistent daily *loss* for a period of three consecutive days, the animal is ready for test. This is known as the depletion period and should require approximately 19 days.

Test

As the animals become ready for test, that is, on the third day after showing a consistent loss of weight, force feed each animal daily by means of the hypodermic syringe, exact amounts of the Cod liver oil to be tested. The animals in the first cage should receive each, .01 cc of Cod liver oil daily. The animals in the second cage should receive .05 cc daily. The animals in the third cage should receive .1 cc daily and the animals in the fourth cage should receive .2 cc daily. Accurate growth records must be kept daily for a period of five weeks. *The cage of animals showing a regular gain of three grams per week have received, per day, the amount of cod liver oil which contains one unit of vitamin A.* Upon measuring the specific gravity of the Cod liver oil this volume figure must be converted into a weight figure and the number of units of vitamin A per gram can then be calculated.

To cite a typical example to illustrate the above calculation, let us assume that each of the rats in one of the cages received daily .02 cc of the Cod liver oil in question and that they showed a regular gain of three grams per week. In the volume of cod liver oil that has been fed them ***each day,*** there is contained one unit of vitamin A. If the specific gravity of the Cod liver oil in question happens to be 0.9, then the weight of Cod liver oil is 0.018—this being the weight of cod liver oil that contains one unit of vitamin A. In order, then, to determine the number of units of vitamin A per gram of the Cod liver oil under test, we divide 1 by 0.018, obtaining as the result 56, which is the number of units U.S.P. XI per gram of Cod liver oil. It is unnecessary to report tenths of units per gram of any test substance since there may be as much as 10 units per gram variation due to experimental error.

It may be necessary to reduce the amount of Cod liver oil received per day in order to get an increase in weight of *only* three grams per week in one or more of these groups of animals. It may even be necessary to give the dosage, as outlined above, in the same quantities per *week* instead of daily. This, of course, can be determined within the first three or four days of the test, particularly by observing the growth curve of the animals on test. If the smallest dosage produces a growth of more than three grams in the first week, reduce the dosage proportionately for all animals and continue the test for five more weeks, selecting the group that shows a gain of three grams weekly per animal.

Chapter IX

SUGGESTIONS FOR ADDITIONAL USES FOR ALBINO RATS

In order to conduct only one or two nutrition experiments one must be provided with all of the essential supplies such as cages, perhaps scales, and live animals, the total cost of which may seem somewhat high in relation to the number of experiments performed. Our contention is that even without further justification the original expense is not exorbitant—that the resulting good to the students is difficult to estimate in terms of dollars and cents but is translated into better health ideas. However if one wishes to get the greatest good out of the equipment it can be used almost constantly for other purposes and we are pleased merely to suggest the other uses.

The two control rats (provided you use four animals in the experiment as we strongly recommend) will obviously be in good condition at the close of the experiment. If they are about twenty eight days old at the start of the experiment and it runs for about a month, perhaps a little more, then it will be seen that they will breed at just about this time unless separated. Keep the male and female in separate cages until they are from 90 days on up to 120 days of age and they may then be mated. This will be a good lesson for elementary schools in the unit on reproduction. The young will become the pets of the entire classroom.

Above, you will notice, we referred to the control animals. But, in the event recovery of the test animals is complete—at least to all external appearances—it might be well to keep them for further work also. Do not, however, cross them with the controls for building up a small colony. If they are bred, keep this stock entirely separate from that of the control animals. An interesting project for the deficient animals would be to prepare a skeleton from one of them and to compare it with the skeleton from a rat, same age, that had received the complete diet all of its life. The difference will not be so marked if complete recovery was accomplished as if the animal were maintained on a deficient diet for a much longer time. Work of this type should not be attempted by students of less than high school standing and may even be more in line with college biology.

Introductory experiments in animal psychology utilize the rat as a suitable test animal. Some of the classical maze learning experiments would certainly be in order in a college biology course. A small colony could be developed with these experiments in mind. One is referred to the text "Principles of Animal Psychology" by Maier and Schneirla, published by McGraw Hill Book Co., if he has an interest in this type of study.

Mammalian tissues for histological study are constantly available when there is maintained a small colony of rats. Sections of rat ovary and testis to show respectively ovogenesis and spermatogenesis are classical examples of slides that will be found useful in any histology course.

The subject of inheritance must not be overlooked in this connection. Mendelian laws as applied to mammals are vividly exemplified by crossing these rats of the pure Albino strain with, for example, the Japanese hooded rat. There is much literature on the subject of inheritance in rats and references can be found without much difficulty. Introductory studies on inheritance using fruit flies are always preferable because of their short life cycle permitting many generations within the short time devoted to this topic. However by supplementing with a mammal the application of Mendelian principles to the human is more easily made by young students.

Embryology, bacteriology, physiology and pathology all find the rat a suitable laboratory experimental animal. There is no end to the usefulness of the rat in any major branch of the biological sciences.

REFERENCES

Teaching Nutrition to Boys and Girls.—Rose. Published by The MacMillan Co.

The Science of Nutrition.—Lusk. Published by The W. B. Saunders Co.

Breeding and Care of the Albino Rat for Research Purposes.—Greenman & Duhring. Published by Wistar Institute.

Introduction to Physiological Chemistry.—Bodansky. Published by John Wiley & Son.

Practical Methods in Bio-Chemistry.—Koch. Published by The William Wood & Co.

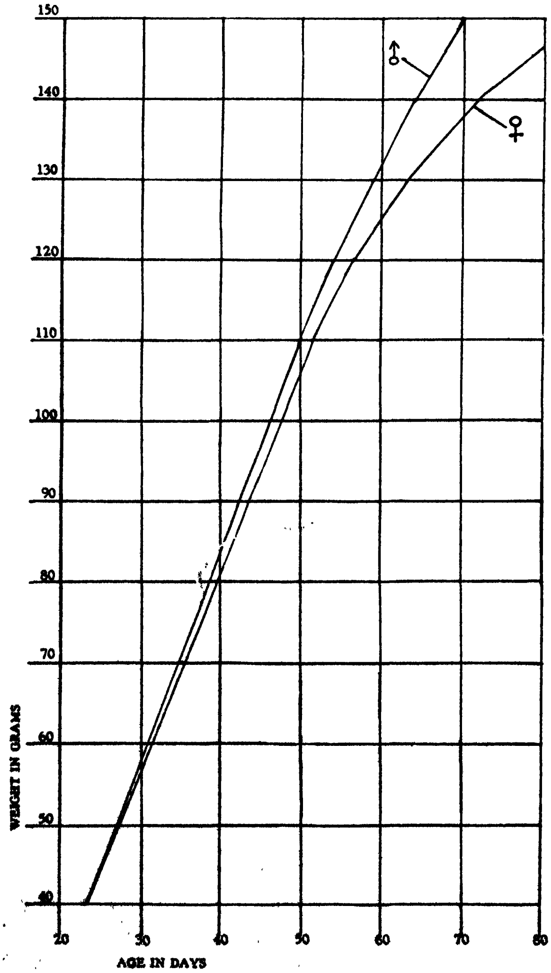
The Rat—Data and Reference Tables.—Donaldson. Published by Wistar Institute.

The Oestrous Cycle in the Rat and Its Associated Phenomena.—Long and Evans. Published by University of California Press.

Laboratory Handbook for Dietetics.—Rose. Published by The MacMillan Co.

Foundations of Nutrition.—Rose. Published by The MacMillan Co.

A Text Book of Biological Assays.—Pittenger. Published by P. Blakiston's Sons & Co.



This is a typical chart for plotting the growth curve on experimental animals. The curves shown here are typical of healthy animals on a complete diet. It is best to subdivide a chart of this kind into individual days and grams where the animals are on feeding tests.

Chapter X

SUPPLIES FOR NUTRITION EXPERIMENTS

ANIMALS

13V74S **Albino Rats. For Breeding Purposes.** Weighing 120 grams or more; at least 50 days of age. Of a pure healthy stock carefully selected for breeding purposes. These animals will breed relatively soon after becoming accustomed to their new quarters if this be desirable. By using this young stock a much longer breeding period is assured. Guaranteed to be free from defect upon delivery.
Each .... $ 1.20
Per pair 2.15
Per dozen animals............. 11.50

13V741 **Young Albino Rats. For Nutrition Experiments.** These animals are of a select strain from one of the largest rat colonies in this country. Specimens of from 25 to 28 days of age are supplied—exact age will be shown on our invoice. Living delivery is guaranteed.
Per two animals. Specify sex... 2.50
Per dozen animals.............. 12.50

13V761 **Young Guinea Pigs. For Vitamin C Experiments.** Weigh between 300 and 350 grams. From healthy well nourished stock and entirely suitable for vitamin C work.
Each ....$ 3.00
Per two animals. Specify sex... 5.00

13V72 **Mice. White.** Mature animals of pure strain but not pedigreed. Suitable for breeding or feeding experiments.
Dozen 10.00
Pair (male and female)......... 2.00

(Write for special quotation of prices on any of the above animals when desired in quantities larger than indicated here. Other animals for nutritional research can be supplied promptly.)

DIETS

55V311 **Protein Deficient Diet Components Set. Unmixed.** Consists of corn starch, sodium chloride, calcium carbonate and yeast, in separate packages, but each in the proper proportion for preparing 3 pounds of the diet according to our formula. The lard and Cod liver oil should be obtained locally. Mixing directions are given. Price for set of these four ingredients...... 1.50

55V3111 **Protein Deficient Diet. Mixed. Ready for Use.** Prepared according to formula given on page 16.
Per lb. .... .60
Per 3 lb. lot.................... 1.70

55V3112 **Protein Sufficient (control) Diet Components Set. Unmixed.** Consists of milk, corn starch, sodium chloride, calcium carbonate and yeast, in separate packages, but each in the proper proportion for preparing 3 pounds of the diet according to our formula. Cod liver oil should be obtained locally. Directions for mixing are given. Price per set of five ingredients.............. 1.70

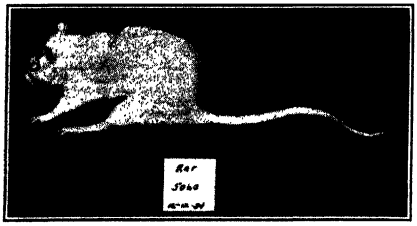
55V3113 **Protein Sufficient (control) Diet. Mixed. Ready for Use.** Prepared according to our regular formula.
Per lb .... .65
Per 3 lb. lot................... 1.80

55V312 **Carbohydrate Deficient Diet Components Set. Unmixed.** Consists of casein, cellu-flour, sodium chloride, calcium carbonate, and yeast, in separate packages and each in the proper quantity for preparing 3 pounds of our standard diet. Butter and Cod liver oil to be obtained locally. Full directions for mixing are included. Price per set of five ingredients. 1.90

55V3121 **Carbohydrate Deficient Diet. Mixed. Ready for Use.** Prepared according to formula on page 17.
Per lb. .... .70
Per 3 lb. lot................... 2.00

55V3122 **Carbohydrate Sufficient (control) Diet Components Set. Unmixed.** Same formula as diet No. 55V3132.
Per set of ingredients for preparing 3 pounds of the mixture........ 1.90

SUPPLIES



Specimen shows malnutrition due to poor protein in diet and nutritional inbalance. Note the humped back and tumor on the eye. Opposite illustration shows same individual, recovered.

55V3123 Carbohydrate Sufficient (control) Diet. Mixed. Ready for Use.
Per lb. $0.70
Per 3 lb. lot 2.00

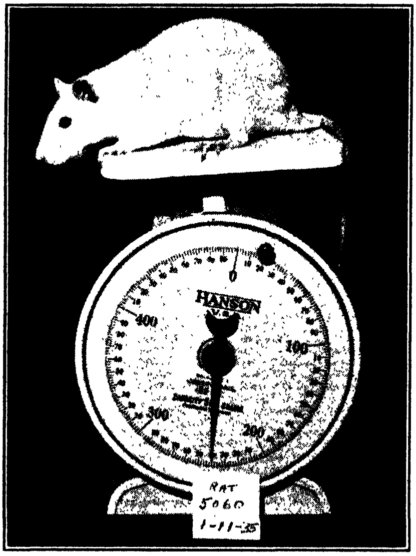
55V313 Fat Deficient Diet Components Set. Unmixed. Consists of casein, corn starch, sodium chloride, calcium carbonate and yeast, in separate packages and each in the proper quantity for preparing 6 pounds of our standard diet. Cod liver oil to be obtained separately. Full directions are given. (The addition of butter to approximately half of this formula makes the **Fat Sufficient [control] Diet.)**
Price per set of ingredients...... 3.25

55V3131 Fat Deficient Diet Components Set. Mixed. Ready for Use. Prepared according to formula on page 18.
Per lb. .... .60
Per 3 lb. lot.................... 1.65

55V3132 Fat Sufficient (control) Diet. Mixed. Ready for Use. Prepared according to formula on page 19.
Per lb. .... .70
Per 3 lb. lot.................... 2.00

55V314 Mineral Deficient Diet Components Set. Unmixed. Consists of casein, corn starch, agar-agar, and yeast (the lard and Cod liver oil should be obtained locally) all in separate packages, but each in the proper proportion for mixing 6 pounds of the diet according to the formula. Full directions are given. (The addition of Salts No. 51, Cod liver oil and lard to one half of this mixture makes the **Mineral Sufficient Diet** for the control group.) Price per set of ingredients................ 3.15

55V3141 Mineral Deficient Diet. Mixed. Ready for Use. Prepared according to formula on page 20.
Per lb. .... .65
Per 3 lb. lot.................... 1.80



Same animal as that to left, after 30 days on a complete diet. Recovery is practically complete.

55V3142 Mineral Sufficient (control) Diet. Mixed. Ready for Use. Same as above except formula is adjusted to contain slightly less of some ingredients plus Salt No. 51.
Per lb. $0.75
Per 3 lb. lot.................... 2.00

55V315 Vitamin A Deficient Diet Components Set. Unmixed. Consists of casein, yeast, corn starch, calcium carbonate and sodium chloride (the cholesterol for this diet should be bought separately in units listed below), each in a separate package and in the proper proportion for mixing 6 pounds of the diet. Full directions are given. (The addition of Cholesterol and Cod liver oil to one half of this formula makes the **Vitamin A Sufficient [control] Diet.)** Price per set of the five ingredients. 3.10

55V3151 Vitamin A Deficient Diet. Mixed. Ready for Use. Prepared according to formula on page 22.
Per lb. .... .65
Per 3 lb. lot.................... 1.70

55V3152 Vitamin A Sufficient (control) Diet. Mixed. Ready for Use. Same as above except it contains in addition, Cod liver oil.
Per lb. .... .75
Per 3 lb. lot.................... 2.10

NUTRITION EXPERIMENTS

55V316 Vitamin B (B_1) Deficient Diet Components Set. Unmixed. Consists of casein, sucrose, Salts No. 51, and autoclaved yeast (the Cod liver oil should be purchased locally), each in a separate package and in the proper proportion for mixing 6 pounds of the diet. Full directions are given. (The addition of dried yeast to one half of this formula makes the **Vitamin B Sufficient [control] Diet.**) Price per set of four ingredients **$3.60**

55V3161 Vitamin B Deficient Diet. Mixed. Ready for Use. Prepared according to formula on page 24.
Per lb. **.70**
Per 3 lb. lot **2.00**

55V3162 Vitamin B Sufficient (control) Diet. Mixed. Ready for Use. Same as above except it contains dried yeast instead of autoclaved yeast.
Per lb. **.65**
Per 3 lb. lot **1.70**

55V3171 Vitamin C Deficient Diet. Mixed. Ready for Use. Prepared according to formula on page 26.
Per lb. **.60**
Per 5 lb. lot **2.75**

All of the prices given in Chapter X are current January 1, 1937, and are subject to change without notice. Current quotations will be submitted upon request.

55V318 Vitamin D Deficient Diet Components Set. Unmixed. Consists of yellow corn meal, gluten flour, sodium chloride and calcium carbonate; in packages, but each in the proper proportion for preparing 6 pounds of the diet according to our formula. Directions for mixing are given. (The addition of Viosterol to one half of this mixture makes the **Vitamin D Sufficient Diet** for the control animals.) Price for set of four ingredients **$3.15**

55V3181 Vitamin D Deficient Diet. Mixed. Ready for Use. Prepared according to formula on page 27.
Per lb. **.60**
Per 3 lb. lot **1.65**

55V3182 Vitamin D Sufficient (control) Diet. Mixed. Ready for Use. Same as above except it includes Viosterol.
Per lb. **.70**
Per 3 lb. lot **2.00**

55V3191 Vitamin G (B_2) Deficient Diet. Mixed. Ready for Use. Contains casein, starch, white corn meal, lard, cod liver oil and Salts No. 51 in proper proportions.
Per lb. **.60**
Per 3 lb. lot **1.65**

55V3192 Vitamin G (B_2) Sufficient (control) Diet. Mixed. Ready for Use. Same as above except it contains a source for Vitamin G.
Per lb. **.75**
Per 3 lb. lot **2.15**

MISCELLANEOUS DIET COMPONENTS

The grade supplied is satisfactory for usual nutrition experiments. Refer to "Diet Grade" when ordering.

	Per Lb.
White corn meal	**$0.20**
Yellow corn meal	**.20**
Ground oats	**.25**
Gluten flour	**.70**
Middlings	**.15**
Bran	**.25**
Barley-meal	**.15**
Yeast (brewer's dried)	**.70**
Autoclaved yeast	**1.50**
Wheatsworth flour (100% whole wheat flour) Vitamin content same as whole wheat grain	**.20**
Corn starch	**.25**
Cellu-flour	**.60**
Fish meal	**.40**
Sucrose	**.30**
Dried Milk powder	**.50**
Casein	**.45**
Agar-agar	**.35**

	Per Lb.
Sodium chloride	**$0.30**
Calcium carbonate	**.35**
Rice polishings	**.20**
Unpolished Rice	**.30**

55V321 Viosterol. Supplied in special dropper top bottle.
Per 5cc bottle **.65**
Per 50cc bottle **3.65**

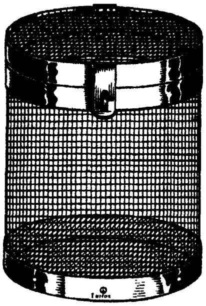
55V322 Salts No. 51.
Per ¼ lb. bottle **.65**

55V323 Irradiated commercial Cholesterol.
Per ¼ lb. bottle **3.00**

Other components such as butter, lard, Cod liver oil, lemons, etc. specified in some of these introductory experiments can best be procured locally.

SUPPLIES

APPARATUS



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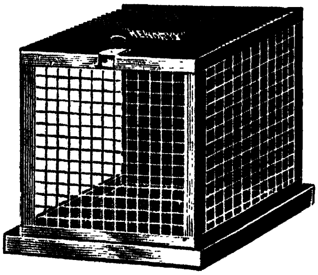


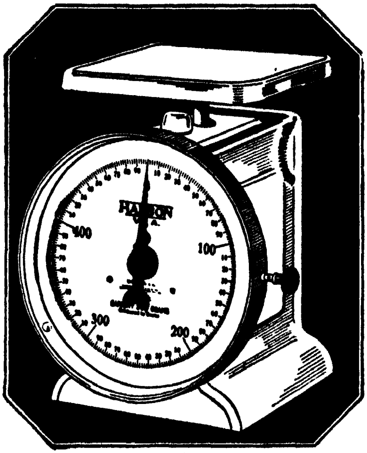
220A44

220A43 Turtox Special Rat Cage. Designed especially for feeding experiments. Made of galvanized wire, fine mesh sides and top, bottom of ½" mesh to permit instant passage of feces. Round, 8½" high, 9¾" in diameter. Hinged top, outside friction latch; built in bottom.
Each $4.00
Per unit of two cages............ 7.50

220A431 Turtox Cage Assembly Set. By using these "knocked-down" parts the teacher can have the students actually construct their own cages. All parts are cut to proper size for bolting together to form a cage similar to above. Directions for assembly are included.
Per complete set................ 3.25

220A432 Enameled Pan. For use with above cages. 13¼" in diameter and 2¾" deep.
Each .... .35

220A44 Hendryx Rat Cage. Square type, 9" x 9" x 9⅛". Made of ½" mesh galvanized wire. Hinged top, open bottom. Cage rests in detached galvanized pan. Supplied with one wire mat ⅛" mesh and one card holder for 3" x 5" data card.
Each 5.50



220A60

220A581 Card Holder. For cages. A stiff metal frame to hold a record or label card. Made with removable transparent cover on the front and hook on back for attaching to cages.
Each, complete with one plain card $0.30
Per dozen 2.75

220A551 Turtox Glass Water Fountain. For attachment to outside of cage. Composed of a two ounce bottle into which a one-hole rubber stopper holding a curved watering tip is inserted. Constant source of water is provided which cannot be contaminated.
Each .... .55

220A581 Turtox Diet Cup Non-Scattering. Prevents the waste of the carefully prepared rations thus eliminating needless expense. Consists of zinc (non-rusting) cup, 3" high by 2¾" in diameter into which fits a metal collar in which there is a small hole for the animal's head only to project through in feeding. Collar can be removed for instant cleaning.
Each 1.25

220A60 Hanson Small Animal and Diet Scale. Capacity 500 grams, graduated in grams. This platform scale is highly recommended for all food experiments, because of its scientific accuracy and the ease with which it can be used. Thumb screw adjustment allows for tare instantly—no computation necessary. A live rat can be weighed in an instant. The platform top is 5½" square—of white porcelain; rotating dial 6¼" in diameter, glass protected. Outside dimensions 6½" x 6½" x 8".
Each 11.00

DATE OF ISSUE

This book must be returned within 3, 7, 14 days of its issue. A fine of ONE ANNA per day will be charged if the book is overdue.